REMARKS/ARGUMENTS

Claim 1 has been revised to expressly denote an inherent feature of the claim which is directed to the detection of the presence of a ligand on cells stained and captured from a cell or tissue sample. Support is provided at least by the claim as filed and the specification at page 4, first and second paragraphs.

Claims 2 and 4 have been rewritten in independent form including all the limitations of claim 1 from which they depend.

No new matter has been introduced, and entry of the above claims is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 103(a)

Claims 1-3, 6-11 and 19 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Reiter et al. and Fend et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

As an initial matter, Applicants point out that the assertion of certain Reiter et al. teachings against claims 2 and 9 in the statement of the rejection appears to be misplaced. A brief review of Reiter et al. demonstrates how the disclosure therein is limited to the use of a centromere 8 probe (directly labeled with SpectrumRed™) and a *MYC* probe (directly labeled with SpectrumGreen™) or *PSCA* probe (labeled with digoxigen-11-dUTP via nick translation) where all of these probes are *nucleic acid based* such that they perform their function by being complementary to "target DNA" sequences (see page 96, right column, last paragraph). Therefore, and to the extent that the instant rejection is based on the assertion that each of these probes is a "ligand binding agent" as required by the claims, none of these probes is an antibody as required by claims 2 and 9. The instant rejection appears to erroneously assert that the rhodamine-labeled anti-digoxigenin antibody meets the requirements of claims 2 and 9. But that antibody is clearly designed to bind and detect the digoxigen moiety in the PSCA probe rather than binding a "ligand" as required by the claims. Because Fend et al. fail to remedy the above

discussed deficiencies, the instant rejection simply fails to address each element present in claims 2 and 9 as required by the standards set forth at MPEP 2143.03 and the cases cited therein. Accordingly, Applicants respectfully submit that the instant rejection should be withdrawn at least with respect to claims 2 and 9.

With regard to claims 1-3, 6-11, and 19 as rejected by the instant rejection, Applicants point out that the claimed methods require a number of acts that are interrelated. Act One is the contacting of a cell or tissue sample with a binding agent, attached to a detectable nucleic acid molecule, which agent binds a ligand in the sample. Act Two is the staining of the sample to identify cells of interest, which are captured or isolated. Act Three is the detection of the detectable nucleic acid molecule *in the captured or isolated cells* to indicate the presence of the ligand. These acts of the claimed methods are in sharp contrast to the limited disclosures in Reiter et al. and Fend et al., the combination of which is misplaced for the following reasons.

The disclosure of Reiter et al. relates to fluorescence *in situ* hybridization, or FISH. This is evident on pages 96 and 97 within the passages most heavily relied upon in the instant statement of the rejection. There are, however, at least two key distinctions between FISH and the claimed methods. First, and quite obviously, a FISH based method is conducted ***in situ***, which in Latin refers to "in the original context or place". Consistent with this meaning, Reiter et al. provide no teaching or suggestion of isolating cells from their original tissue architecture as found in a tissue sample. Stated differently, there is no teaching, suggestion, or other indication of capturing cells from a larger extracellular context in which the cells are found in a sample. This is in sharp contrast to the instant claims, which requires isolation or capture of cells. Thus, and in sharp distinction to the disclosure of Reiter et al., the claimed methods relate to removing cells from their *in situ* context.

Second, and because there is no isolation of cells, Reiter et al. fail to teach or suggest the staining of cells in a sample to facilitate their isolation or capture. The instant statement of the rejection refers to page 96, right column, second paragraph for the proposition that Reiter et al. teach the use of hematoxylin and eosin (H&E) staining. A careful review of that paragraph, however, reveals that of 15 tissue section slices for each case, only the first section of each 15 was so stained to "ascertain the region of interest". There is no teaching or suggestion

that the same section was used for FISH, and certainly no teaching or suggestion of isolating or capturing cells from that section. This failure to teach or suggestion cell isolation or capture remains even if, assuming *in arguendo*, the same stained section was used for FISH.

Thus Reiter et al. fail to disclose or suggest at least Acts Two and Three as required by the instant claims.

The statement of the rejection appears to recognize the above deficiencies in Reiter et al. by admitting that "Reiter et al. did not teach capturing or isolating stained cells". But Applicants respectfully submit that Reiter et al.'s deficiencies are greater than that. Beyond failing to teach or suggest the capture or isolation of stained cells, Reiter et al. also do not teach the staining of cells that have been contacted with any of their probes. They also do not teach or suggest *the detection of their probes in any captured or isolated cell.*

The above deficiencies of Reiter et al. are not remedied by the disclosure of Fend et al. Fend et al. disclose the process of "immuno-LCM", where cells of a sample are immunostained followed by their isolation by laser capture microdissection (LCM) for *mRNA analysis*. But given the nature of the immuno-LCM process and *the focus on obtaining cells for mRNA analysis*, there is no teaching, suggestion, or other indication of detecting an agent bound ligand *after* cell isolation via LCM. This is in contrast to the instant claims, where the act of detecting the presence of a ligand *after* cell isolation is an express part of the claimed methods. Moreover, and in sharp distinction to Reiter et al., Fend et al. is clearly directed to the isolation of cells out of their *in situ* context. Thus the instant rejection must be based at least in part on the assertion that it would be obvious to modify the *in situ* based methods of Reiter et al. via the method of Fend et al. to **remove** cells from *in situ* architecture.

Evidence of this assertion in the rejection was presented as follows:

> "it would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made, to combine a method of detecting a ligand in a tissue or cell sample as taught by Reiter et al. with the step of capturing or isolating the cells of interest as taught by Fend et al. to achieve expected advantage of developing a sensitive and enhanced method for detecting a cell

specific ligand because Fend et al. taught that the immunohistochemical staining technique for tissue section , in combination with capturing cells by laser capture microdissection allows very fast, easy and precise isolation of specific cell populations for mRNA analysis...."

But the above assertion clearly does not address how the methods of Reiter et al. and Fend et al. can be combined to arrive at Acts One, Two and Three of the claims as discussed above. While the instant rejection may be based on the argument that Fend et al.'s immuno-LCM process corresponds to Act Two, where is the guidance or suggestion to perform the Fend et al. process in the middle of Acts One and Three, which have components that correspond to Reiter et al.'s FISH based process? Stated differently, the statement of the rejection provides no basis for how the actions in the processes of Reiter et al. and Fend et al. are to be rearranged and combined to meet the requirements of the instant claims, where a cell containing sample, after contact with "a ligand detecting agent" attached to a "detectable nucleic acid molecule" (Act One); is stained to identify cells of interest followed by capture or isolation of cells (Act Two); before detection of the detectable nucleic acid molecule in the captured or isolated cells to indicate the presence of the ligand (Act Three).

As the Office no doubt recognizes, a *prima facie* case of obviousness against a claimed method requires more than the mere identification of all the actions of the method in various references. In addition to a motivation for the combination, a basis for how the actions in disparate methods are to be combined in a manner and order as encompassed by the claimed invention is needed. Otherwise, the assertion of obviousness would be based upon impermissible hindsight reconstruction using the patent applicant's own disclosure as a guide. Applicants respectfully submit that no *prima facie* case of obviousness has been presented because impermissible hindsight was used given the absence of an explanation regarding why the artisan of ordinary skill would modify the FISH based process of Reiter et al. by insertion of the immuno-LCM based process of Fend et al., while maintaining *the detection of the FISH ligand in the LCM isolated cells.*

Moreover, the statement of the rejection also does not address why the artisan of ordinary skill would modify the *in situ* based process of Reiter et al. to become a non-*in situ* process based upon the isolation acts of Fend et al. The instant statement of the rejection asserts that motivation is present

> "to combine the method of Reiter et al. with the incorporation of the step of capturing or isolating specific cells of interest as taught by Fend et al. to improve the sensitivity and specificity of the detection method [of Reiter et al.] because the skilled artisan would have a reasonable expectation of success that the inclusion of capturing or isolating cells of interest would result in isolating or capturing specific cell populations from a highly heterogenous primary tissue, such as tumor samples, by eliminating unwanted cell populations which would facilitate further characterization of these specific cells."

Applicants respectfully submit that the above is inadequate to meet the motivation required to support a *prima facie* case because 1) it attempts to combine Reiter et al.'s *in situ* based process with the Fend et al. non-*in situ* process without providing motivation for why an artisan of ordinary skill would combine disparate processes; 2) it fails to address why, by implication, an artisan of ordinary skill would omit the mRNA preparation and analysis portion of a combination of the Reiter et al. and Fend et al. processes; and 3) it attempts to bolster the alleged motivation by asserting that a "reasonable expectation of success" is present in the combination. As the Office is no doubt aware, the requirements for an adequate motivation to combine and a reasonable expectation of success are distinct requirements where one cannot substitute for the other. See the distinctions drawn between MPEP 2143.01 and 2143.02 and the cases cited in each. In light of the failure to provide an adequate motivation for the combination, the instant rejection is misplaced and should be withdrawn.

Finally, and with respect to the asserted expectation of success, Applicants respectfully submit that the above quote appears to be a paraphrasing of the disclosure in the

instant application rather than based upon the teachings or suggestion of the cited references. This is evident at least in light of Fend et al.'s focus on isolating cells for mRNA analysis rather than for facilitating the detection of a ligand in some cells of a tissue sample. Moreover, Reiter et al. do not teach or suggest any problem or difficulty in the ability to use their FISH based process to detect ligand signals in a tissue sample. To the contrary, Reiter et al.'s disclosure is generally based upon the successful use of their process to detect ligands in complex samples containing cells *in situ*. Therefore, and in the absence of the instant application, why would an artisan of ordinary skill be concerned about needing to eliminate "unwanted cell populations ... to facilitate further characterization of [] specific cells" as asserted in the instant rejection? Applicants respectfully submit that the instant rejection cannot be based upon a reasonable expectation of derived from Applicants own disclosure. Withdrawal of the rejection on this basis is also urged.

Claims 1-2, 4-5, 13-15, and 18-21 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Eberwine et al. (USP 5,922,553) and Fend et al. (as discussed above). Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

As an initial matter, Applicants point out that the Eberwine et al. disclosure fails to meet requirements recited in the rejected claims. For example, Eberwine et al. fail to describe the staining or isolation of any cell, which is consistent with its disclosure of "cell contents harvested using solid phases" and "harvesting of cell media alone" (see column 5, lines 5-8, and columns 6-7, Example 6, as well as column 4, lines 20-24). To the extent that the statement of the rejection relies upon Fend et al. to remedy this deficiency, Applicants respectfully submit that the combination of Eberwine et al.'s cell destroying process and Fend et al.'s cell isolating process is misplaced as addressed further below.

Moreover, and contrary to the statement of the rejection, there is no disclosure of PCR amplification in relation to the invention disclosed by Eberwine et al.[1] The cited passages at column 6, lines 35-52, and column 5, lines 9-24, are directed to the use of RNA amplification using an RNA polymerase. This is wholly distinct from the use of PCR and quantitative PCR as recited in claims 4 and 5. Also contrary to the statement of the invention, there is also no disclosure of the use of a microarray as recited in claim 20. Because Fend et al. fail to remedy these deficiencies with respect to claims 4, 5, and 20, the instant rejection simply fails to address each element present in these claims as required by the standards set forth at MPEP 2143.03 and the cases cited therein. Accordingly, Applicants respectfully submit that the instant rejection should be withdrawn at least with respect to claims 4, 5 and 20.

With regard to claims 1-2, 4-5, 13-15, and 18-21 and as noted above with respect to Reiter et al. and Fend et al., the combination of Eberwine et al.'s cell destroying process and Fend et al.'s cell isolating process is misplaced because the combination fails to lead to the claimed invention and because no adequate motivation to combine has been presented. Simply put, Eberwine et al. disclose a process (best reviewed at column 3, line 50 to column 4, line 4; and column 4, lines 17-36) which is very different from the claimed invention.

To demonstrate the differences, Applicants again point out that the claimed methods require Acts One through Three as described with respect to the combination of Reiter et al. and Fend et al. above. These acts of the claimed methods are in sharp contrast to the limited disclosures in Eberwine et al. and Fend et al. For example, Eberwine et al. describe the "harvest" of "protein from a single cell" (see column 6, lines 57-58). The cellular contents were aspirated into a patch pipette coated with Tau-1 antibody (see column 6, lines 60-63). The pipette was then treated as described in Examples 4 and 5 (column 6) by an incubation, rinsing, and crushing of the pipette tip into an eppendorf tube followed by contact with a cDNA tagged Tau-1 antibody and subsequent RNA amplification of the cDNA to detect the presence of Tau-1.

As would be obvious to the skilled person, Eberwine et al. fails to teach or suggest contact between a cell containing sample with a "ligand binding agent" as required by the instant claims. Simply put, the disrupted contents of a cell is not a "cell" as encompassed by

[1] The only discussion of PCR is in Eberwine et al.'s "Background" section.

the instant claims. Eberwine et al. only discloses contacting a cDNA tagged antibody with cellular contents, not intact cells. Moreover, this contacting is only after certain cellular contents have been immobilized on a solid support like a "siliconized patch pipettes, microtiter plates and beads" (see column 4, lines 17-20) and "rinsed". Given the absence of intact cells, it is entirely logical that Eberwine et al. fail to disclose the staining and isolation of cells as required by the claims. Therefore, Eberwine et al. at least fail to disclose or suggest Acts Two and Three as required by the instant claims.

The statement of the rejection appears to recognize the above deficiencies in Eberwine et al. by admitting that "Eberwine et al. did not teach capturing or isolating stained cells". But as pointed out above with respect to Reiter et al. and Fend et al., Applicants respectfully submit that Eberwine et al.'s deficiencies are greater than that. Beyond not teaching or suggesting the capturing or isolating of stained cells, Eberwine et al. also do not teach the staining of cells that have been contacted with any of their cDNA tagged antibodies. They also do not teach or suggest *the detection of their antibodies in any captured or isolated cell.*

The above deficiencies of Eberwine et al. are not remedied by the disclosure of Fend et al., which relate to immuno-LCM as explained above. Again, and given the nature of the immuno-LCM process and *the focus on obtaining cells for mRNA analysis*, there is no teaching, suggestion, or other indication of how the isolation of cells from an *in situ* context, as disclosed by Fend et al., are to be combined with the cellular contents manipulation as disclosed by Eberwine et al. Thus the instant rejection must fail at least because of the absence of how the teachings of the two references can be combined to result in the claimed invention.

Based upon the above described limitations of the cited references and the failure to demonstrate how the ordinary artisan would have combined them to result in the claimed invention, the assertion of a *prima facie* case in the instant rejection, which is identical to that in the rejection based on Reiter et al. and Fend et al., is misplaced. Simply put, the assertion clearly does not address how the methods of Eberwine et al. and Fend et al. can be combined to arrive at Acts One, Two and Three of the claims as discussed above. While Fend et al.'s immuno-LCM process may be argued as corresponding to Act Two, where is the guidance or suggestion to precede the Fend et al. process with Act One and follow the Fend et al. process with Act Three?

Moreover, where are the cells of Act One when Eberwine et al. teach the contacting of their cDNA tagged antibodies with immobilized cellular contents rather than cells? Additionally, where are the cells to be stained for Act Two and the cells for detection of ligand in Act Three? Stated differently, the statement of the rejection provides no basis for how the actions in the processes of Eberwine et al. and Fend et al. are to be rearranged and combined to meet the requirements of the instant claims, where a cell containing sample, after contact with "a ligand detecting agent" attached to a "detectable nucleic acid molecule" (Act One); is stained to identify cells of interest followed by capture or isolation of cells (Act Two); before detection of the detectable nucleic acid molecule in the captured or isolated cells to indicate the presence of the ligand (Act Three).

Moreover, the statement of the rejection also does not address why the artisan of ordinary skill would modify the *cell contents* based process of Eberwine et al. by inclusion of steps from a *cell isolation* based process as disclosed by Fend et al. The instant statement of the rejection asserts that motivation is present

> "to combine the method of Eberwine et al. with the incorporation of the step of capturing or isolating specific cells of interest as taught by Fend et al. to improve the sensitivity and specificity of the detection method [of Eberwine et al.] because an ordinary person skilled in the art would have a reasonable expectation of success that the inclusion of the step of capturing or isolating cells of interest would result in isolating or capturing specific cell populations from a highly heterogenous primary tissue, such as tumor samples by eliminating unwanted cell populations, which would facilitate further characterization of these specific cells."

Applicants respectfully submit that the above is inadequate to meet the motivation required to support a *prima facie* case because 1) it attempts to combine Eberwine et al.'s cell contents based process with the Fend et al. cell isolation based process without providing motivation for why an artisan of ordinary skill would combine these disparate processes; and 2)

it attempt to bolster motivation by assertion that a "reasonable expectation of success" is present in the combination. As noted above, the requirements for both an adequate motivation to combine and a reasonable expectation of success are distinct requirements as set out at MPEP 2143.01 and 2143.02 and in the cases cited therein. In light of the failure to provide an adequate motivation for the combination, the instant rejection is misplaced and should be withdrawn.

Finally, and with respect to the asserted expectation of success, Applicants respectfully submit that the above quote again appears to be a paraphrasing of the disclosure in the instant application rather than based upon the teachings of the cited references. For example, Eberwine et al. do not teach or suggest any problem in the ability to use their cell contents based process to detect ligand signals. To the contrary, they emphasize how their technique works with the contents of a single cell (see for example, column 4, lines 21-25). Therefore, and in the absence of the instant application, why would an artisan of ordinary skill be concerned about needing to eliminate "unwanted cell populations ... to facilitate further characterization of [] specific cells" as asserted in the instant rejection? As noted above, Eberwine et al. are already working with the contents of single cells, which also argues for why the combination with Fend et al.'s cell isolation process is misplaced. Again, Applicants respectfully submit that the instant rejection cannot be based upon a reasonable expectation of derived from Applicants own disclosure. Withdrawal of the rejection on this basis is also urged.

Claims 16 and 17 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Reiter et al. in light of Fend et al. and Wang et al. Applicants have carefully reviewed the statement of the rejection as well as the cited references. Applicants respectfully traverse because no *prima facie* case of obviousness has been presented.

The deficiencies and failings of the combination of Reiter et al. and Fend et al. as noted above are again present with respect to the instant rejection because Wang et al. fail to correct any of the deficiencies and failings. Accordingly, this rejection should be withdrawn for the same reasons as that provided with respect to Reiter et al. and Fend et al. above.

Moreover, claims 16 and 17 are dependent from claim 8 which requires the use of a plurality of agents to detect a plurality of ligands. Wang et al., however, only disclose the use

of a single antibody against AR to detect phosphorylated and unphosphorylated forms via Western Blotting (see pages 23-24, bridging paragraph). Thus there is no plurality of ligand binding agents as required by the claims, and this rejection fails to address all the requirements of the claims as required for a *prima facie* case.

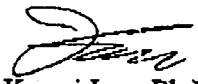
In light of the above, this rejection is misplaced and should be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 858-350-6100.

Respectfully submitted,



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